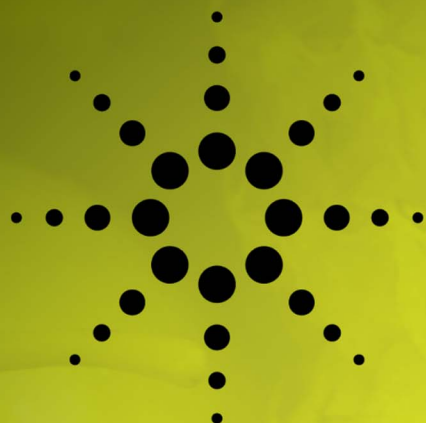
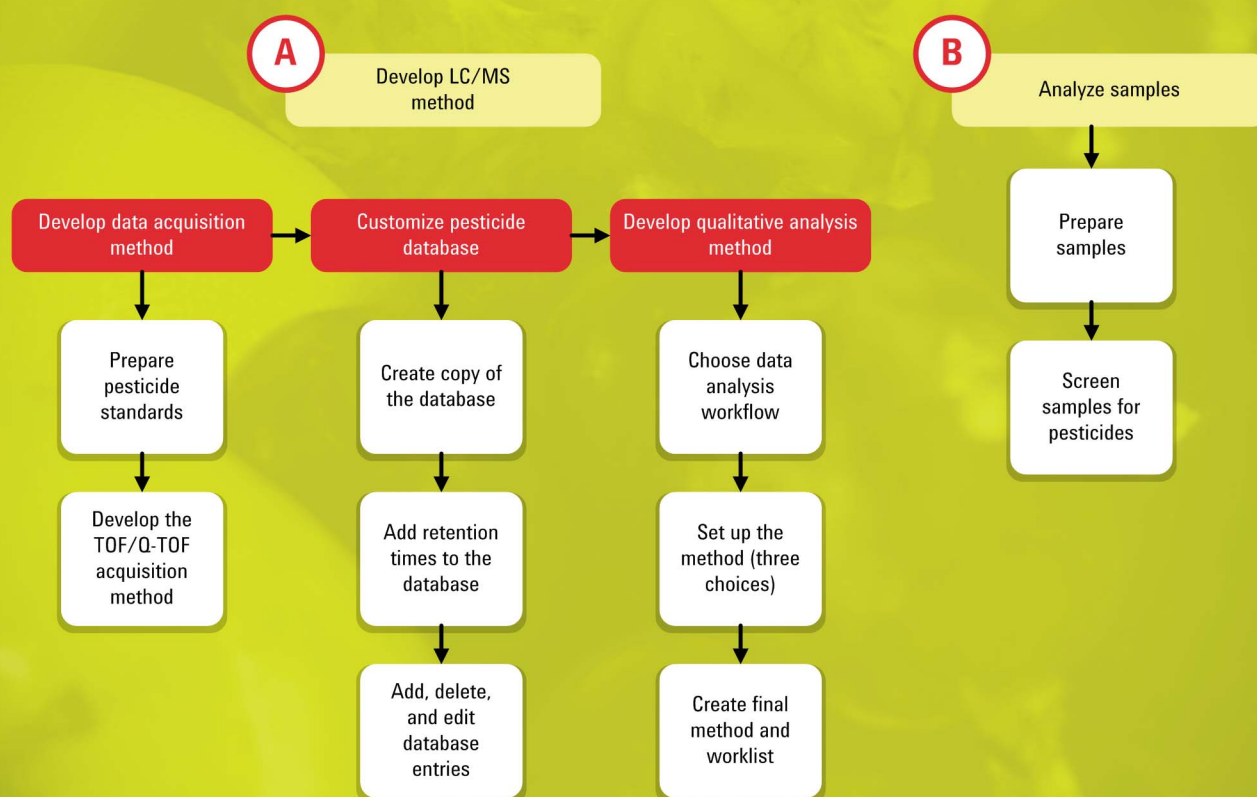




Agilent TOF LC/MS and Q-TOF LC/MS Screening of Pesticides

Workflow Overview



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Required Items

Required hardware and software



Figure 1 The workflow requires an Agilent LC and TOF or Q-TOF LC/MS System.

To do this workflow, you need:

- One of the following LCs:
 - Agilent 1220 Infinity LC
 - Agilent 1260 Infinity LC
 - Agilent 1290 Infinity LC
 - Agilent 1200 Series LC system
 - Agilent 1200 Series Rapid Resolution LC system
- One of the following mass spectrometers:
 - Agilent 6200 Series Accurate-Mass TOF LC/MS System
 - Agilent 6500 Series Accurate-Mass Q-TOF LC/MS System
- Agilent MassHunter software:
 - Agilent MassHunter Data Acquisition software for TOF/Q-TOF version B.02.01
 - Agilent MassHunter Qualitative Analysis software version B.03.01 SP3
 - Agilent MassHunter Personal Pesticide Database version B.03.01
 - Agilent MassHunter Personal Compound Database and Library (PCDL) software version B.03.01 (which is installed when you install the Personal Pesticide Database)

Optional kit

The Agilent MassHunter Personal Pesticide Database Kit (G6854AA) is very helpful for this analysis. To save method development time, the kit contains the database and PCDL software, as well as pesticide test mixes, an Agilent ZORBAX Eclipse Plus C18 column, a trial Agilent SampliQ QuEChERS sample preparation kit, and valuable documentation, methods, and data files.

Developing the LC/MS Data Acquisition Method

The workflow includes method development and sample analysis. See the *Agilent TOF LC/MS and Q-TOF LC/MS Screening of Pesticides Workflow Guide* for details about the steps in this *Workflow Overview*.

Prepare pesticide standards

1. Do preliminary work.

- a Choose an initial LC/MS method for data acquisition.
- b Purchase standards and solvents, if you have not already done so.

2. Prepare the standards.

- a Prepare stock standards of individual pesticides.
- b Prepare a test mix of pesticides to test the data acquisition and data analysis methods that you will develop.
- c Prepare retention time standards that you will use to determine retention times for targeted analysis, or to determine retention times of selected pesticides for quality assurance purposes.
- d Prepare additional standards required to ensure that your method works properly, including standards used to spike samples/determine recoveries, as well as internal standards.
- e Refrigerate the standards.

Develop the TOF/Q-TOF data acquisition method

1. Prepare the LC/MS system.

- a Prepare LC solvents.
- b Start the MassHunter Data Acquisition program.
- c Prepare the LC modules.
- d Prepare the Agilent TOF or Q-TOF.



2. Set up LC/MS parameters.

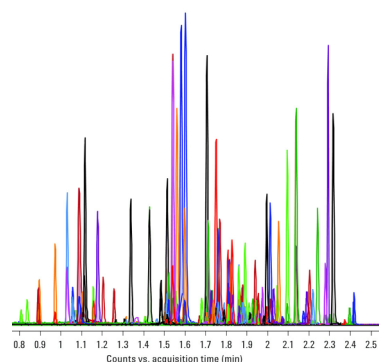
- a Enter values for all the LC modules.
- b Enter TOF or Q-TOF MS parameters.
- c Save the method.

3. Acquire data for test mixture of pesticides.

- a Open MassHunter Data Acquisition, if it is not already open.
- b Put the test mix standard into the LC autosampler.
- c Load the method you created in “[Set up LC/MS parameters.](#)”
- d Set up to analyze the test mix (sample name, data file, etc.).
- e Start the run.

4. View results and adjust method, if necessary.

- a Open MassHunter Qualitative Analysis.
- b Open the data file you created in the last task.
- c Use the MS Target Compound Screening workflow.
- d Find compounds by formula.
- e Display the results to see if all the pesticides were found.
- f If some pesticides were not found, do the following, in order, until you find the pesticides:
 - Change the Find by Formula settings, if necessary.
 - Use extracted ion chromatograms (EICs) to search for the pesticides.
 - Run the analysis again, but inject more sample or prepare and analyze a standard at higher concentration.
 - Investigate other reasons such as poor ionization.

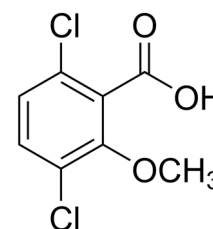


Add retention times to the database

1. Analyze pesticides to get retention times.
2. Export retention times from data files.
3. Update the pesticide database with retention times.

- a Open the MassHunter Personal Compound Database and Library (PCDL) software.
- b Create a copy of the Agilent MassHunter Personal Pesticide Database, so you can customize it (**File > New Database/Library**).

- Open MassHunter Data Acquisition, if it is not already open.
- Put the retention time standards into the LC autosampler.
- Set up a worklist to analyze the retention time standards.
- Run the worklist.



- a Open Open MassHunter Qualitative Analysis.
- b Use the MS Target Compound Screening workflow.
- c Make sure retention times use minutes as units.
- d Save the method with a new name.
- e Determine the spectral noise level, for use in the molecular feature extractor (MFE).
- f Set up and use MFE to find all the compounds in the data file. See the *Workflow Guide* for details.
- g Export the mass list to a CSV file. (If you have only one data file with retention times, it may be easier to copy and paste the mass list into the PCDL software.)
- h Repeat [step e](#) through [step g](#) for any additional data files that contain the retention times you need.

- 5

Add, delete, and edit database entries

- c Click the **Batch Search** tab.
- d Open a CSV file that you created in "[Export retention times from data files.](#)"
- e Set the search parameters.
- f Start the search.
- g View hits for selected masses.
- h Resolve any results that conflict (indicated in red).
- i Click the **Batch Summary** tab.
- j Update retention times in the database from the current mass list.
- k Repeat [step c](#) through [step j](#) for any additional CSV files that you created.

-
- a Open the MassHunter Personal Compound Database and Library (PCDL) software, and your custom pesticide database.
 - b Enable editing for this database.
 - c Edit the database.



Developing the Qualitative Analysis Method

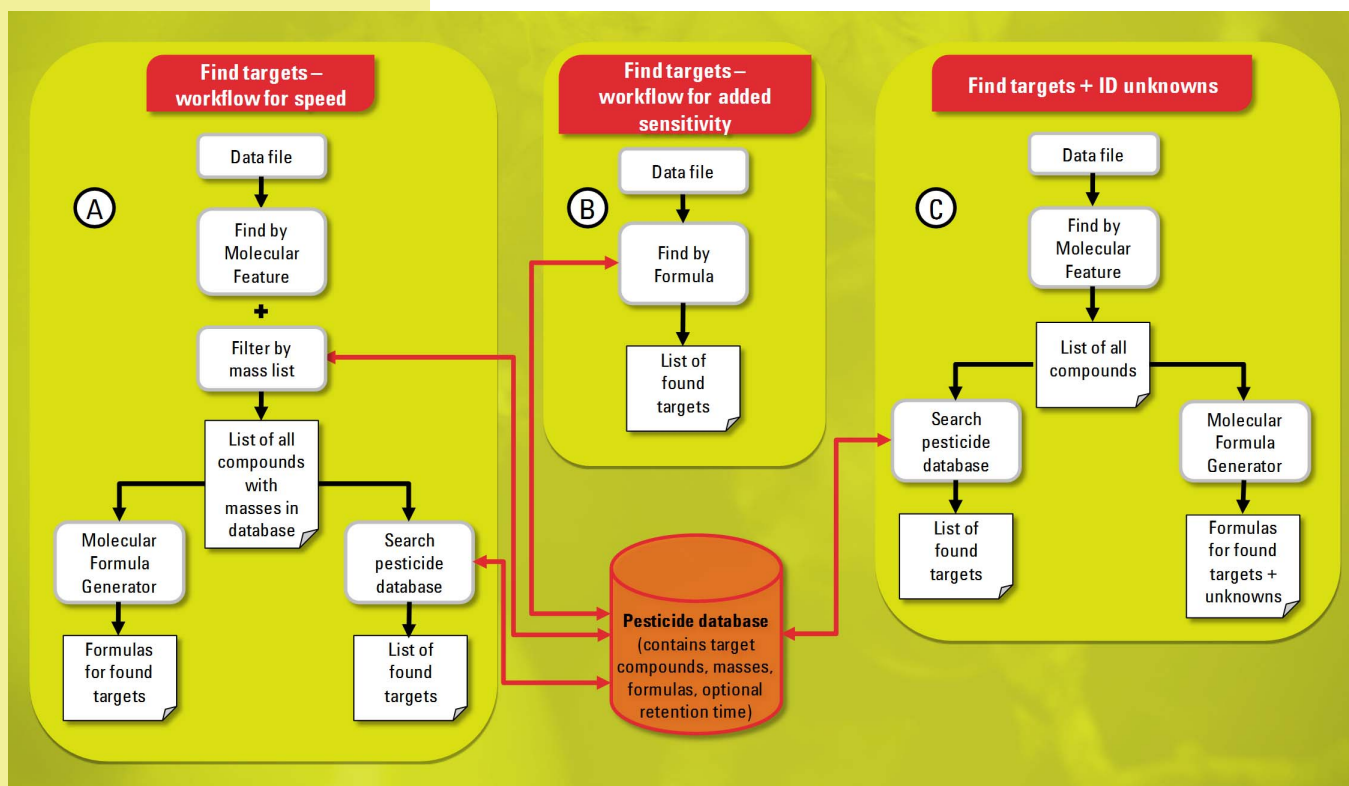
Choose a data analysis workflow

1. Learn about the workflows.

- Read the *Workflow Guide* to learn more about the analysis approaches shown below.

2. Pick a workflow.

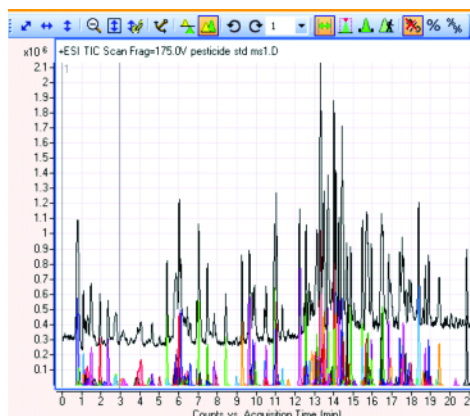
- Considering your lab's needs, choose one of the workflows shown below to analyze the data.



Set up the data analysis method (three choices)

Option A: Find by Molecular Feature Extraction (MFE)

- Open MassHunter Qualitative Analysis.
- Use the MS Target Compound Screening workflow.
- Save the method with a new name.
- Open the data file that you acquired for your test mix of pesticides and observe the spectral noise level.
- Set parameters for Find by Molecular Feature:
 - Extraction tab
 - Ion Species tab
 - Compound Filters tab
 - Mass Defect tab
 - Mass Filters tab – (Filter mass list by masses in the pesticide database.)
 - Results tab
 - Charge State tab
- Set parameters for database search.
- Set the report options.
- Set parameters for automation.
- Save the method.
- Test the method.
- Adjust the method, if necessary.
- Test the method again, and save when you are done.



Option B: Find by Formula

- Open MassHunter Qualitative Analysis.
- Use the MS Target Compound Screening workflow.
- Save the method with a new name.
- Open the data file that you acquired for your test mix of pesticides.
- Set parameters for Find by Formula:
 - Formula Source tab
 - Formula Matching tab
 - Positive Ions or Negative Ions tab
 - EIC Peak Filters tab
 - Results tab

Data Navigator	
Sort by Data File	
☒	Cpd 11: Butopyronoxyl
☒	Cpd 12: Bentazone
☒	Cpd 13: Etofen
☒	Cpd 14: Carbofuranphenol
☒	Cpd 15: Dibutyl succinate
☒	Cpd 16: Imazalil Degradate
☒	Cpd 17: Flupropanate
☒	Cpd 18: 2-Phenoxybenzoic acid
☒	Cpd 19: Tricamba
☒	Cpd 20: 2,4,5-T
☒	Cpd 21: Chlorprocarb
☒	Cpd 22: Silvex
☒	Cpd 23: Proglazine-ethyl
☒	Cpd 24: Picric acid
☒	Cpd 25: Azinphos-methyl
☒	Cpd 26: Nitrofluorfen
☒	Cpd 27: Acifluorfen
☒	Cpd 28: Citronellal hydrate

Option C: Find by MFE plus formula generation for unknowns

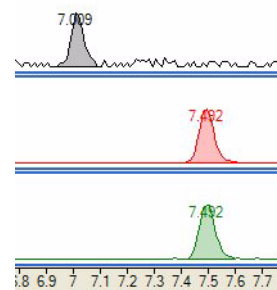
Create final method and worklist

Option A: Add the data analysis method to the data acquisition method

Option B: Run data acquisition and data analysis as separate methods

- f Set the report options.
- g Set parameters for automation.
- h Save the method.
- i Test the method.
- j Adjust the method, if necessary.
- k Test the method again, and save when you are done.

- a Open MassHunter Qualitative Analysis.
- b Use the MS Target Compound Screening workflow.
- c Save the method with a new name.
- d Set up the method similar to “Option A: Find by Molecular Feature Extraction (MFE)”.
 - Follow [step d](#) through [step l](#), except in [step e](#), in the Mass Filters tab, do *not* filter the MFE mass list by the masses in the pesticide database.



- a Open your data analysis method.
- b Save the data analysis method within the data acquisition method.
- c In MassHunter Data Acquisition, create a worklist that runs the combined method.
- d Save the worklist.

- a Set up a worklist that uses both methods.
- b Save the worklist.

Analyzing Samples

Prepare samples



•Follow the applicable QuEChERS method, or another sample preparation method you have chosen. If you need to select a QuEChERS method, see “Agilent SampliQ QuEChERS Kits” (Agilent publication [5990-3562EN](#)).

Screen samples for pesticides

- a Make sure you have all the standards and samples ready to go in the appropriate containers for your LC autosampler.
- b Put the standards and samples into the LC autosampler.
- c Start the MassHunter Data Acquisition program.
- d Set up a worklist to perform a completely automated screening analysis.
- e Run the analysis.
- f Review the reports that are automatically generated.



For More Information

For details about these procedures, see the *Agilent TOF LC/MS and Q-TOF LC/MS Screening of Pesticides Workflow Guide*.

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